

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (currently amended) An *in vitro* protein or nucleic acid synthesis system comprising:

at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease, wherein said E. coli cell does not express Gam; ~~and~~, wherein said at least one extract is modified by the addition of Gam protein. -
2. (withdrawn) The *in vitro* synthesis system according to claim 1, wherein the at least one extract from a cell has reduced activity of at least one nuclease.
3. (withdrawn) The *in vitro* synthesis system according to claim 1, wherein the at least one extract from a cell has reduced activity of at least one phosphatase.
4. (withdrawn) The *in vitro* synthesis system according to claim 1, wherein the at least one extract from a cell has reduced activity of at least one polymerase.
5. (canceled)
6. (withdrawn) The *in vitro* synthesis system according to claim 1, wherein the at least one inhibitor inhibits at least one phosphatase.
7. (withdrawn) The *in vitro* synthesis system according to claim 1, wherein the at least one inhibitor inhibits at least one polymerase.

8. (withdrawn) The *in vitro* synthesis system according to claim 2, wherein the template is a DNA template and the nuclease is a DNase.

9. (withdrawn) The *in vitro* synthesis system according to claim 8, wherein the DNase is a DNA exonuclease.

10. (withdrawn) The *in vitro* synthesis system according to claim 8, wherein the DNase is a DNA endonuclease.

11. (withdrawn) The *in vitro* synthesis system according to claim 10, wherein the DNA endonuclease is endonuclease A.

12. (withdrawn) The *in vitro* synthesis system according to claim 2, wherein the nuclease is an RNase.

13. (withdrawn) The *in vitro* synthesis system according to claim 12, wherein the RNase is an RNA exonuclease.

14. (withdrawn) The *in vitro* synthesis system according to claim 12, wherein the RNase is an RNA endonuclease.

15. (withdrawn) The *in vitro* synthesis system according to claim 14, wherein the endonuclease is RNase E.

16. (original) The *in vitro* synthesis system according to claim 1, further comprising at least one nucleic acid template selected from the group consisting of a DNA template and an RNA template.

17. (original) The *in vitro* synthesis system according to claim 16, comprising at least one DNA template and wherein the *in vitro* synthesis system is an *in vitro* transcription/translation system.

18. (withdrawn) The *in vitro* synthesis system according to claim 3, wherein the phosphatase is an alkaline phosphatase.

19. (withdrawn) The *in vitro* synthesis system according to claim 1, wherein the at least two energy sources generate or regenerate high energy triphosphate compounds.

20. (withdrawn) The *in vitro* synthesis system according to claim 1, comprising at least one or more compounds selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

21. (withdrawn) The *in vitro* synthesis system according to claim 1, wherein the at least one extract from said cell is reduced in activity of at least one enzyme selected from the group consisting of OmpT, RNase E, alkaline phosphatase and endonuclease I.

22. (withdrawn) The *in vitro* synthesis system according to claim 21, further reduced in at least one activity selected from the group consisting of RNase I or RNase I*.

23. (withdrawn) The *in vitro* synthesis system according to claim 1 comprising at least two energy sources providing chemical energy for synthesis.

24. (withdrawn) The *in vitro* synthesis system according to claim 23, further comprising at least one extract from a cell having reduced activity of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase.

25. (withdrawn) The *in vitro* synthesis system according to claim 23, further comprising at least one inhibitor of at least one enzyme selected from the group consisting of a nuclease, a polymerase and a phosphatase.

26. (withdrawn) The *in vitro* synthesis system according to claim 23, further comprising at least one inhibitor of at least one enzyme selected from the group consisting of a nuclease, a phosphatase and a polymerase.

27. (canceled)

28. (currently amended) The *in vitro* synthesis system according to claim 1, wherein said Gam protein is a soluble Gam protein.

29. (canceled)

30. (previously presented) The *in vitro* synthesis system according to claim 1, comprising at least one energy source.

31. (withdrawn) The *in vitro* synthesis system according to claim 23, wherein the at least one energy source comprises at least two different energy sources, each of which generates or regenerates high energy triphosphate compounds for synthesis.

32. (withdrawn) The *in vitro* synthesis system according to claim 31, wherein the at least two different chemical fuel sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

33. (withdrawn) The *in vitro* synthesis system according to claim 32, wherein the at least two different chemical fuel sources comprise at least PEP and acetyl phosphate.

34. (withdrawn) The *in vitro* synthesis system according to claim 1, comprising said at least one extract, at least one nucleic acid template and at least one energy source.

35. (canceled)

36. (withdrawn) The *in vitro* synthesis system according to claim 1, comprising at least one nucleic acid template and said at least two energy sources.

37. (withdrawn) The *in vitro* synthesis system according to claim 1, comprising said at least one extract.

38-40 (canceled)

41. (currently amended) A kit for *in vitro* synthesis comprising:

at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease, wherein said E. coli cell does not express Gam[[]], wherein said at least one extract is modified by the addition of Gam protein[[]]; and

one or more nucleotides or derivatives thereof, one or more amino acids or derivatives thereof, one or more polymerases, one or more cofactors, one or more buffers or buffer salts, one or more energy sources, one or more nucleic acid templates, or one or more reagents to determine the efficiency of the kit or assay.

42. (canceled)

43. (withdrawn) A method for producing protein or nucleic acid from a nucleic acid template in an *in vitro* system comprising:

contacting said template with at least one component selected from the group consisting of:

at least one extract from a cell having reduced activity of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds;

at least one inhibitor of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds; and

at least two energy sources providing chemical energy for synthesis,
to form a mixture; and

incubating said mixture under conditions sufficient to produce at least one protein encoded by said template.

44. (withdrawn) The method according to claim 43, wherein the at least one enzyme is selected from the group consisting of OmpT, RNase E, alkaline phosphatase and endonuclease I.

45. (withdrawn) The method according to claim 43, wherein the inhibitor is Gam.

46. (withdrawn) The method according to claim 41, wherein each of the at least two energy sources generates or regenerates high energy triphosphate compounds for protein synthesis.

47. (withdrawn) The method according to claim 46, wherein the at least two energy sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

48. (withdrawn) The method according to claim 43, wherein said enzyme is selected from the group consisting of a nuclease, a phosphatase and a polymerase.

49. (withdrawn) A method for constructing an *in vitro* synthesis system, said method comprising:

obtaining at least one cell extract;

mixing the cell extract with one or more components selected from the group

consisting of:

at least one inhibitor of at least one enzyme that catalyzes hydrolysis of high energy phosphate bonds or hydrolysis or formation of phosphodiester bonds; and

at least two energy sources providing chemical energy for synthesis.

50. (withdrawn) The method according to claim 49, wherein said at least one enzyme is selected from the group consisting of a nuclease, a phosphatase and a polymerase.

51. (currently amended) A composition comprising:

at least one extract from an E. coli cell having a mutation that results in reduced activity of at least one nuclease, wherein said E. coli cell does not express Gam; ~~and, wherein~~ said at least one extract is modified by the addition of Gam protein, and

at least one nucleic acid template in the presence of at least a partial synthesis product of said template.

52. (original) The composition according to claim 51, wherein the product is a nucleic acid product.

53. (original) The composition according to claim 52, wherein the nucleic acid product is a DNA.

54. (original) The composition according to claim 52, wherein the nucleic acid product is a RNA.

55. (previously presented) The *in vitro* synthesis system of claim 30, comprising at least two energy sources.

56. (canceled)

57. (previously presented) The kit of claim 41, comprising at least two energy sources.

58-59 (canceled)

60. (previously presented) The composition of claim 51, further comprising at least two energy sources providing chemical energy for synthesis.

61. (currently amended) The *in vitro* protein or nucleic acid synthesis system of claim 1, wherein said nuclease [[in]] is a DNase.

62. (previously presented) The *in vitro* protein or nucleic acid synthesis system of claim 61, wherein said DNase is exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV.

63. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 62, wherein said DNase is endonuclease I.

64. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 62, wherein said DNase is endonuclease A.

65. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 1, wherein said nuclease is an RNase.

66. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 65, wherein said RNase is endoribonuclease I (RNase I), endoribonuclease M, endoribonuclease R, endoribonuclease III, endoribonuclease P, endoribonuclease E (RNase E), endoribonuclease K, endoribonuclease H, endoribonuclease HII, endoribonuclease IV, endoribonuclease F, endoribonuclease N, endoribonuclease P2, endoribonuclease O, endoribonuclease PC, endoribonuclease PIV, polynucleotide phosphorylase, oligoribonuclease, exoribonuclease II, exoribonuclease D, exoribonuclease BN, exoribonuclease T, exoribonuclease PH or exoribonuclease R.

67. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 66, wherein said RNase is endoribonuclease I (RNase I).

68. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 66, wherein said RNase is endoribonuclease E (RNase E).

69. (currently amended) The kit of claim 41, wherein said nuclease [[in]] is a DNase.

70. (previously presented) The kit of claim 69, wherein said DNase is exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV.

71. (withdrawn) The kit of claim 70, wherein said DNase is endonuclease I.

72. (withdrawn) The kit of claim 71, wherein said DNase is endonuclease A.

73. (withdrawn) The kit of claim 41, wherein said nuclease is an RNase.

74. (withdrawn) The kit of claim 73, wherein said RNase is endoribonuclease I (RNase I), endoribonuclease M, endoribonuclease R, endoribonuclease III, endoribonuclease P, endoribonuclease E (RNase E), endoribonuclease K, endoribonuclease H, endoribonuclease HII, endoribonuclease IV, endoribonuclease F, endoribonuclease N, endoribonuclease P2, endoribonuclease O, endoribonuclease PC, endoribonuclease PIV, polynucleotide phosphorylase, oligoribonuclease, exoribonuclease II, exoribonuclease D, exoribonuclease BN, exoribonuclease T, exoribonuclease PH or exoribonuclease R.

75. (withdrawn) The kit of claim 74, wherein said RNase is endoribonuclease I (RNase I).

76. (withdrawn) The kit of claim 74, wherein said RNase is endoribonuclease E (RNase E).

77. (currently amended) The composition of claim 51, wherein said nuclease [[in]] is a DNase.

78. (previously presented) The composition of claim 77, wherein said DNase is exonuclease I, exonuclease II, exonuclease III, exonuclease IVA, exonuclease IVB, RecBCD (exonuclease V), exonuclease VII, exonuclease VIII, RecJ, dRpase, endonuclease I, endonuclease III, endonuclease IV, endonuclease V, endonuclease VII, endonuclease VIII, endonuclease A, fpg, uvrABC, mutH, vsr endonuclease, ruvC, ecoK, ecoB, mcrBC, mcrA, mrr, topoisomerase I, topoisomerase II, topoisomerase III, or topoisomerase IV.

79. (withdrawn) The composition of claim 78, wherein said DNase is endonuclease I.

80. (withdrawn) The composition of claim 78, wherein said DNase is endonuclease A.

81. (withdrawn) The composition of claim 51, wherein said nuclease is an RNase.

82. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 81, wherein said RNase is endoribonuclease I (RNase I), endoribonuclease M, endoribonuclease R, endoribonuclease III, endoribonuclease P, endoribonuclease E (RNase E), endoribonuclease K, endoribonuclease H, endoribonuclease HII, endoribonuclease IV, endoribonuclease F, endoribonuclease N, endoribonuclease P2, endoribonuclease O, endoribonuclease PC, endoribonuclease PIV, polynucleotide phosphorylase, oligoribonuclease, exoribonuclease II, exoribonuclease D, exoribonuclease BN, exoribonuclease T, exoribonuclease PH or exoribonuclease R.

83. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 82, wherein said RNase is endoribonuclease I (RNase I).

84. (withdrawn) The *in vitro* protein or nucleic acid synthesis system of claim 82, wherein said RNase is endoribonuclease E (RNase E).

85. (previously presented) The *in vitro* synthesis system according to claim 55, wherein each of the at least two different energy sources generates or regenerates high energy triphosphate compounds for the synthesis.

86. (previously presented) The *in vitro* synthesis system according to claim 85, wherein the at least two different energy sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

87.(previously presented) The *in vitro* synthesis system of claim 86, wherein two of the at least two energy sources are phosphoenol pyruvate and acetyl phosphate.

88-90 (canceled)

91. (previously presented) The kit of claim 57, wherein each of the at least two different energy sources generates or regenerates high energy triphosphate compounds for the synthesis.

92. (previously presented) The kit of claim 91, wherein the at least two different energy sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

93. (previously presented) The kit of claim 92, wherein two of said at least two energy sources are phosphoenol pyruvate and acetyl phosphate.

94. (previously presented) The composition of claim 60, wherein each of the at least two different energy sources generates or regenerates high energy triphosphate compounds for the synthesis.

95. (previously presented)The composition of claim 94, wherein the at least two different energy sources are selected from the group consisting of pyruvate, phosphoenolpyruvate (PEP), carbamoyl phosphate, acetyl phosphate, creatine phosphate, phosphopyruvate, glyceraldehyde-3-phosphate and glucose-6-phosphate.

96. (previously presented) The composition of claim 95, wherein two of said at least two energy sources are phosphoenol pyruvate and acetyl phosphate.